AMENDMENTS OF THE CLAIMS

In the Claims

The following Listing of Claims, in which deleted text appears as ~~struck-through~~ and inserted text appears underlined, will replace all prior listings, and versions, of claims in the application.

Listing of Claims

Claim 1. (original) A method of analyzing a polynucleotide sample for one or more target sequences, comprising the steps of:

contacting a polynucleotide sample suspected of comprising one or more target sequences with: (i) a first signal probe which is capable of hybridizing to at least a portion of a first target sequence and producing a first detectable signal when hybridized thereto; (ii) a first quencher probe which is capable of hybridizing in quenching proximity to the first signal probe and quenching the signal of the first signal probe when hybridized in quenching proximity thereto, said first quencher probe having a T_m below that of the first signal probe; (iii) at least a second signal probe which is capable of hybridizing to at least a portion of a second target sequence and producing a second detectable signal when hybridized thereto; and (iv) an optional second quencher probe which is capable of hybridizing in quenching proximity to the second signal probe and quenching the signal of the second signal probe when hybridized in quenching proximity thereto, said optional second quencher probe having a T_m below that of the second signal probe;

monitoring the detectable signals of the signal probes as a function of temperature; and

determining therefrom the presence or absence of one or more target sequences in said polynucleotide sample.

Claim 2. (original) The method of Claim 1 in which the first and second detectable signals are fluorescent signals.

Claim 3. (original) The method of Claim 2 in which the first and second fluorescent signals are spectrally resolvable.

Claim 4. (original) The method of Claim 1 in which the T_m of the first signal probe is higher than the T_m of the second signal probe.

Claim 5. (original) The method of Claim 1 in which the T_m of the first quencher probe is in the range of about 5 to 10°C lower than that of the first signal probe and the T_m of the optional second quencher probe is in the range of about 5 to 10°C lower than that of the second signal probe.

Claim 6. (original) The method of Claim 2 in which the first and second fluorescent signals are not spectrally resolvable, and the second signal probe has a lower T_m than the first quencher probe.

Claim 7. (original) The method of Claim 6 in which the T_m, of the first quencher probe is in the range of about 5 to 10°C lower than that of the first signal probe and the T_m of the optional second quencher probe is in the range of about 5 to 10°C lower than that of the second signal probe.

Claim 8. (original) The method of Claim 6 in which the T_m of the second signal probe is in the range of about 7 to 15°C lower than that of the first signal probe.

Claim 9. (original) The method of Claim 6 in which the first and second fluorescent signals are the same.

Claim 10. (currently amended) The method of Claim 1 in which the optional second quencher probe is present.

Claim 11. (original) The method of Claim 1 in which the first and second signal probes are self-indicating signal probes.

Claim 12. (original) The method of Claim 11 in which the self-indicating probes are hairpin probes.

Claim 13. (original) The method of Claim 12 in which the first signal, first quencher, second signal and optional second quencher probes are resistant to degradation by nucleases.

Claim 14. (original) The method of Claim 12 in which the first signal, first quencher, second signal and optional second quencher probes are each, independently of one another, selected from the group consisting of a DNA nucleobase oligomer, an RNA nucleobase oligomer and a PNA nucleobase oligomer.

Claim 15. (original) The method of Claim 14 in which the first signal, first quencher, second signal and optional second quencher probes are all DNA, RNA or PNA nucleobase oligomers.

Claim 16. (original) The method of Claim 11 in which the self-indicating probes are linear self-indicating probes.

Claim 17. (original) The method of Claim 16 in which the first signal, first quencher, second signal and optional second quencher probes are resistant to degradation by nucleases.

Claim 18. (original) The method of Claim 16 in which the first signal, first quencher, second signal and optional second quencher probes are each, independently of one another, selected from the group consisting of DNA, RNA and PNA nucleobase oligomers.

Claim 19. (original) The method of Claim 18 in which the first signal, first quencher, second signal and optional second quencher probes are all DNA, RNA or PNA nucleobase oligomers.

Claim 20. (original) The method of Claim 18 in which the first signal, first quencher, second signal and optional second quencher probes are all PNA nucleobase oligomers.

Claim 21. (original) The method of Claim 11 in which each self-indicating probe includes a label which is capable of distinguishing hybridized from unhybridized signal probe.

Claim 22. (original) The method of Claim 21 in which the label is a fluorescent intercalating dye.

Claim 23. (original) The method of Claim 22 in which the fluorescent intercalating dye is selected from the group consisting of acridine orange, ethidium bromide, propidium iodide, hexium iodide, ethidium bromide homodimer, 3,3'-diethylthiadicarbocyanine iodide, SYBR® Green I and SYBR® Green II 7-aminoactinomycin D, and actinomycin D.

Claim 24. (original) The method of Claim 21 in which the label is a fluorescent minor-groove-binding dye.

Claim 25. (original) The method of Claim 24 in which the fluorescent minor-groove-binding dye is selected from the group consisting of bisbenzimide dyes such as Hoechst 332589, Hoechst 33342, and Hoechst 34580 and indole dyes such as DAPI (4',6-diamino-2-phenylindole).

Claim 26. (original) The method of Claim 1 in which the detectable signals are monitored as a function of decreasing temperature from a temperature above the T_m of the first signal probe to a temperature below the T_m of the optional second quencher probe.

Claim 27. (original) The method of Claim 26 in which the detectable signals are monitored at temperatures approximately equal to the T_ms of the signal and quencher probes.

Claim 28. (original) The method of Claim 26 in which the detectable signals are monitored at temperatures approximately halfway between the T_ms of the signal and quencher probes.

Claim 29. (original) The method of Claim 26 in which the temperature is decreased at a rate in the range of about 0.01 °C/minute to about 5 °C/minute.

Claim 30. (original) The method of Claim 26 in which the detectable signals are monitored continuously at a rate in the range of about every 100 to 10,000 msec as a function of temperature.

Claim 31. (original) The method of Claim 1 in which the detectable signals are monitored as a function of increasing temperature from a temperature below the T_m of the optional second quencher probe to a temperature above the T_m of the first signal probe.

Claim 32. (original) The method of Claim 31 in which the detectable signals are monitored at temperatures approximately equal to the T_ms of the signal and quencher probes.

Claim 33. (original) The method of Claim 31 in which the detectable signals are monitored at temperatures halfway between the T_ms of the signal and quencher probes.

Claim 34. (original) The method of Claim 31 in which the temperature is increased at a rate in the range of about 0.01 °C/minute to about 5 °C/minute.

Claim 35. (original) The method of Claim 31 in which the detectable signals are monitored continuously at a rate in the range of about every 100 to 10,000 msec as a function of temperature.

Claim 36. (original) The method of Claim 1 in which the detectable signals are monitored as a function of temperature by determining the T_ms of the first and second signal probes.

Claim 37. (original) The method of Claim 1 in which the polynucleotide sample is selected from the group consisting of genomic DNA, cDNA, RNA, mRNA, rRNA and an amplification product.

Claim 38. (original) The method of Claim 37 in which the polynucleotide sample is single-stranded.

Claim 39. (original) The method of Claim 1 in which the polynucleotide sample comprises two or more different polynucleotides.

Claim 40. (original) The method of Claim 1 in which the target sequences are present on two or more polynucleotides.

Claim 41. (original) The method of Claim 1 in which the target sequence is present on the same polynucleotide strand.

Claim 42. (original) The method of Claim 1 in which the target sequence is present on two different polynucleotide strands.

Claim 43. (original) A method of analyzing a polynucleotide sample for one or more target sequences, comprising the steps of:

contacting a polynucleotide sample with: (1) a first set of ***m*** signal-quencher probe pairs, each of which comprises (i) a signal probe capable of hybridizing to a portion of a target sequence and producing a first detectable signal when hybridized thereto and (ii) a corresponding quencher probe capable of hybridizing in quenching proximity to the signal probe and quenching its detectable signal when hybridized in quenching proximity thereto, wherein the first signal probe has the highest T_m and the T_m of each quencher probe is lower than the T_m of its corresponding signal probe and the T_m of each signal probe is lower than the T_m of the quencher probe of the preceding signal-quencher probe pair, and further wherein the quencher probe of the signal-quencher probe pair of the first set having the lowest T_m is optional; and (2) a second set of ***n*** signal-quencher probe pairs, each of which comprises (i) a signal probe capable of hybridizing to a portion of a target sequence and producing a second detectable signal distinguishable from the first detectable signal when hybridized thereto and (ii) a corresponding quencher probe capable of hybridizing in quenching proximity to the signal probe and quenching its detectable signal when hybridized in quenching proximity thereto, wherein the T_m of each quencher probe is lower than the T_m of its corresponding signal probe and the T_m of each signal probe is lower than the T_m of the quencher probe of the preceding signal-quencher probe pair,

and further wherein the quencher probe of the signal-quencher probe pair of the second set having the lowest T_m is optional;

monitoring the first and second detectable signals as a function of temperature; and

determining the presence or absence of one or more target sequences in said polynucleotide sample.

Claim 44. (original) A method of genotyping an organism, comprising the steps of:

contacting a polynucleotide sample from the organism, or an amplification product thereof, with a first plurality of signal-quencher probe pairs, each of which is capable of hybridizing, in quenching proximity, to a different genotype-specific sequence and producing a resolvable, temperature-dependent on-off hybridization profile;

obtaining temperature-dependent on-off hybridization profiles for the signal-quencher probe pairs; and

determining therefrom the genotype of the organism.

Claim 45. (original) A method of genotyping a virus, comprising the steps of:

contacting a polynucleotide sample from a virus, or an amplification product thereof, with a first plurality of signal-quencher probe pairs, each of which is capable of hybridizing, in quenching proximity, to a different virus genotype-specific sequence and producing a resolvable, temperature-dependent on-off hybridization profile;

obtaining temperature-dependent on-off hybridization profiles for the signal-quencher probe pairs; and

determining therefrom the genotype of the virus.

Claim 46. (original) A method of analyzing a sample for the presence of a polynucleotide sequence of interest, comprising the steps of:

contacting a polynucleotide from the sample, or an amplification product thereof, with a first plurality of signal-quencher probe pairs, wherein each said signal-quencher probe pair is capable of hybridizing, in quenching proximity, to a different known target sequence and producing a resolvable, temperature-dependent, on-off hybridization profile;

obtaining temperature-dependent, on-off hybridization profiles for the signal-quencher probe pairs; and

determining the presence or absence of one or more different target sequences.

Claim 47. (original) A multiplex method of genotyping a polynucleotide of an organism, comprising the steps of:

amplifying the polynucleotide in the presence of amplification primers suitable for producing a plurality of genotype-specific amplicons and a plurality of signal-quencher probe pairs, wherein each said signal-quencher probe pair is capable of hybridizing, in quenching proximity, to a different genotype-specific amplicon and producing a resolvable, temperature-dependent, on-off hybridization profile;

obtaining temperature-dependent, on-off hybridization profiles for the signal-quencher probe pairs; and

determining therefrom the genotype of the organism.

Claim 48. (original) A multiplex method of diagnosing a patient for a malady of interest, comprising the steps of:

incubating a polynucleotide sample derived from the patient in the presence of a plurality of signal-quencher probe pairs, wherein each said signal-quencher probe pair is capable of hybridizing, in quenching proximity, to a different target sequence indicative of a particular malady of interest and producing a resolvable, temperature-dependent, on-off hybridization profile when hybridized thereto;

obtaining temperature-dependent, on-off hybridization profiles for the signal-quencher probe pairs; and

determining therefrom whether the patient has the malady of interest.

Claim 49. (previously presented) A multiplex method of diagnosing a patient for a malady of interest, comprising the steps of:

amplifying a polynucleotide sample derived from the patient in the presence of amplification primers suitable for producing a plurality of different amplicons, each of which

correlates to a different malady of interest, and a plurality of signal-quencher probe pairs, wherein each said signal-quencher probe pair is capable of hybridizing, in quenching proximity, to a different amplicon and producing a resolvable, temperature-dependent, on-off hybridization profile;

obtaining temperature-dependent, on-off hybridization profiles for the signal-quencher probe pairs; and determining therefrom whether the patient has the malady of interest.

Claims 50-51 (canceled)